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Combined Effects of Chlordiazepoxide Treatment and Food Deprivation on Concurrent Measures of Feeding and Activity

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COLE, S O *Combined effects of chlordiazepoxide treatment and food deprivation on concurrent measures of feeding and activity* PHARMAC BIOCHEM BEHAV 18(3) 369–372, 1983 —The effects of chlordiazepoxide (CDP—0, 5, 10 mg/kg) on feeding, rearing, and ambulatory locomotion of male Holtzman rats were investigated in an open-field arena under 3 different conditions of food deprivation (0, 24, 48 hr) Both CDP and food deprivation enhanced feeding, with their combined effects being essentially additive Also, CDP significantly decreased both rearing and ambulatory locomotion, although this effect tended to be counteracted by increases in food deprivation The interrelationship (correlation) of the behavioral effects of CDP suggested that the reduction in activity measures is due, in part, to the competing or incompatible nature of feeding and that rearing and ambulatory locomotion are somewhat redundant measures of activity under the present conditions

Chlordiazepoxide Food deprivation Hyperphagia Rearing Ambulatory locomotion Interrelationship of drug effects

CHLORDIAZEPOXIDE (CDP) and other benzodiazepine derivatives reliably increase food consumption in experimental animals [2, 9, 12, 13], primarily by increasing total eating duration [5,6] While there remains some uncertainty concerning the mechanism underlying this hyperphagic effect, it appears that the increase in food consumption with CDP is the result of a direct enhancement in feeding motivation (appetite) rather than the drug's anxiolytic action which might indirectly facilitate feeding by a reduction in food neophobia [5, 7, 8, 10] Such a conclusion is based, largely, upon the observation that typical doses of CDP (5 and 10 mg/kg) enhance feeding responses to familiar food without significantly altering responses to novel food [5, 7, 8]

The effect of CDP on activity, however, appears to be much less reliable, in that both decreases in stabilimeter performance [12,13], open-field ambulation [14], and frequency of rearing [11] as well as increases in spontaneous locomotor activity [17,18] have been reported in the literature Apparently, the effects of CDP on general activity depend upon dose and drug history [17], experience with the environment [9] as well as, possibly, how one defines such behavior

Furthermore, the effects of CDP on feeding and activity have, in most instances, been assessed independently of each other (in different observation periods) rather than concurrently (within same observation period) Yet, the assessment of the concurrent effects of another psychoactive drug (d-amphetamine) on feeding and activity has demonstrated that multiple drug actions can compete or be incompatible with each other, thereby altering one's interpretation of the data [3,4] The present study addresses itself to this particular issue in the case of CDP effects as well as further investigating the importance of food deprivation to such effects More specifically, the present study investigates (a) the importance of three levels of food deprivation (0, 24, 48 hr) to the effects of CDP on concurrent measures of feeding and general activity, and (b) the degree of interrelationship (correlation) between the behavioral effects of the drug The second of these objectives is particularly relevant to the issue of competing or incompatible drug-induced behaviors

METHOD

Subjects and Apparatus

Thirty adult, male Holtzman rats (300–450 g) were subjects They were housed individually under standard laboratory conditions and had ad lib access to water in the home cage They were also permitted ad lib access to Purina laboratory chow in the home cage, except when otherwise specified in the food deprivation procedure

An 80×80 cm open-field arena, with walls 30 cm high, served as the test apparatus The floor of the arena was marked off into sixteen 20 cm squares for purposes of scoring ambulatory movement A plastic food cup was firmly attached to one wall of the arena at floor level and permitted free access to food, however, water was not available in the test arena Fluorescent lighting directly above the test area provided uniform illumination of the arena

Procedure

Initially, the 30 subjects were assigned randomly to one of 3 food deprivation groups (0, 24, 48 hr). While under their appropriate food deprivation condition, all subjects were given two 30-min adaptation sessions, separated by approximately 1 week. During adaptation sessions, subjects were permitted to explore the open field and to eat freely 45 mg precision food pellets placed in the food cup.

Following adaptation, all subjects, while again under their appropriate food deprivation condition, were administered three 30-min CDP sessions (0, 5, 10 mg/kg chlordiazepoxide hydrochloride in 1 ml/kg 0.9% NaCl), with the order of drug dose randomly assigned to animals over successive sessions. Approximately 1 week separated the last adaptation session from the first test session and each of the test sessions. Measures of feeding and activity were determined for each test session. Food consumption was measured by placing 250 precision pellets in the food cup at the beginning of the session, counting the number remaining at the end of the session, and taking the difference (corrected for spillage) as the number of pellets eaten. Activity was measured both in terms of the number of discrete rearing events (regardless of duration) and the number of squares entered by subjects. Hand counters were used to record activity data, and a single observer, blind to treatment conditions, recorded all data.

The testing procedure for any one subject was as follows. The animal, after having been food deprived for the specified number of hours (with the exception of the 0 hr deprivation groups), was removed from the home cage, weighed, injected IP with the appropriate dose of CDP, and returned to the home cage. Thirty minutes later, the subject was placed in the open-field test arena, in front of and facing the food dish, to begin the 30-min test session. Upon completion of the session, the animal was returned immediately to the home cage.

RESULTS

The effect of CDP doses on the food consumption of subjects in the different food deprivation groups is summarized in panel A of Fig. 1. Overall analysis (ANOVA) of these data demonstrated a highly significant enhancement of food consumption by CDP: Drug Dose effect, $F(2,54)=68.46$, $p<0.01$. While there was also a significant enhancement of feeding by food deprivation: Deprivation effect, $F(2,27)=6.48$, $p<0.01$, the Drug Dose × Deprivation Interaction was not significant, suggesting that the combined effects of the treatment conditions were essentially additive in nature. Further analysis of drug dose data (t-test for related samples following ANOVA, minimum 0.05 level of significance) in both the 0 and 48 hr groups indicated that the 10 mg/kg dose differed significantly from the 5 mg/kg dose and the vehicle (0 mg/kg), however, the 5 mg/kg dose did not differ from vehicle. In the 24 hr deprivation group, both drug doses differed significantly from vehicle and from each other.

The effect of CDP doses on the rearing of subjects in the different food deprivation groups is summarized in panel B of Fig. 1. Overall analysis (ANOVA) of these data demonstrated a significant CDP-induced reduction in rearing: Drug Dose effect, $F(2,54)=27.71$, $p<0.01$, and a deprivation-induced enhancement of rearing: Deprivation effect, $F(2,27)=3.62$, $p<0.05$, which tended to counteract the effectiveness of the drug. The Drug Dose × Deprivation Interaction, however, was not statistically significant. Further

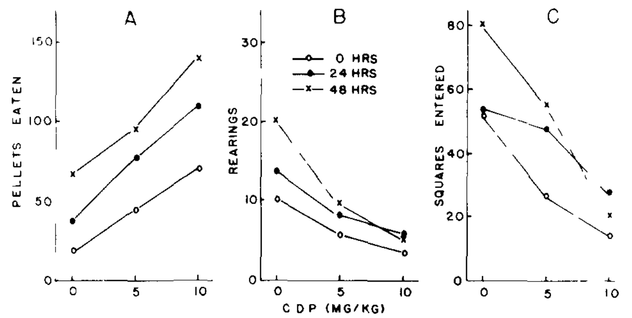

FIG. 1. Mean number of pellets eaten (panel A), mean number of rearings (panel B) and mean number of squares entered (panel C) by subjects in 0, 24 and 48 hr food deprivation groups receiving CDP (0, 5, 10 mg/kg) treatment. Drug doses were assigned randomly to subjects over three 30-min test sessions.

analysis of Drug Dose data (t-test for related samples following ANOVA, minimum 0.05 level of significance) indicated that under 0 hr deprivation the 5 and 10 mg/kg doses differed from vehicle but not from each other. Under 24 and 48 hr deprivation, the 10 mg/kg dose differed from both the 5 mg/kg dose and vehicle, with the 5 mg/kg dose also differing from vehicle under 48 hr deprivation.

The effect of CDP doses on the ambulatory locomotion (squares entered) of subjects in the different food deprivation groups is summarized in panel C of Fig. 1. While this measure yielded higher activity scores, the general pattern of a significant CDP-induced reduction in ambulatory locomotion: Drug Dose effect, $F(2,54)=32.11$, $p<0.01$, and a deprivation-induced enhancement of ambulatory locomotion: Deprivation effect, $F(2,27)=3.35$, $p<0.05$, was similar to that observed with rearing. Again, the Drug Dose × Deprivation Interaction did not reach statistical significance. Further analysis of Drug Dose data (t-test for related samples following ANOVA, minimum 0.05 level of significance) indicated that under 0 hr deprivation the 5 and 10 mg/kg doses differed from vehicle but not from each other. Under 24 and 48 hr deprivation, the 10 mg/kg dose differed from both the 5 mg/kg dose and vehicle, with the 5 mg/kg dose also differing from vehicle under 48 hr deprivation.

To further determine the interrelationship of CDP effects on the concurrent measures of behavior, drug-dose correlations of feeding with rearing, feeding with ambulatory locomotion, and rearing with ambulatory locomotion were calculated for each subject in the different food deprivation groups. The individual correlations of subjects within each group were then pooled to form a deprivation-group composite correlation, with these results summarized in Table 1. As is apparent, the effects of CDP on feeding demonstrated a progressively significant negative correlation with the effects of the drug on rearing and ambulatory locomotion (squares entered) with an increase in food deprivation. In contrast, the effects of CDP on rearing and ambulatory locomotion demonstrated a significant positive correlation under all deprivation conditions, with only a slight change in correlation with an increase in food deprivation.

DISCUSSION

The results of the present study demonstrate the effec-

TABLE 1

SUMMARY OF DRUG DOSE CORRELATIONS (PEARSON r) OF FEEDING WITH REARING (F-R), FEEDING WITH AMBULATORY LOCOMOTION (F-A), AND REARING WITH AMBULATORY LOCOMOTION (R-A) IN 0, 24, AND 48 HR FOOD DEPRIVATION GROUPS

	Food Deprivation Groups (hr)		
Comparisons	0	24	48
F-R	−0 5561	−0 6593*	−0 7989†
F-A	−0 5744	−0 6577*	−0 7952†
R-A	+0 7786†	+0 7942†	+0 8084†

*0 05 Level of significance ($df=8$)
†0 01 Level of significance ($df=8$)

tiveness of CDP in enhancing food consumption in both sated (0 hr) and food deprived (24 and 48 hr) animals and are consistent with previous findings of a similar nature [9] While the assessment of food consumption in the present case does not resolve the controversy as to whether the action of CDP on feeding is a direct one (enhancement of feeding motivation) or an indirect one (anxiolytic action), the results tend to favor the former interpretation Such a conclusion is based upon two specific facts First, the subjects were adapted (for two 30-min sessions) to the food prior to assessing drug effects, thereby minimizing the importance of a reduction in food neophobia to the enhancement of feeding Secondly, the effects of CDP and food deprivation on feeding were additive as was indicated by the absence of a significant interaction between treatment conditions, suggesting that they enhance feeding by similar means Since food deprivation directly enhances feeding motivation, the same, then, might be aruged for the effect of CDP In view of the fact that modification of GABA receptor properties in the CNS appears to be a major mediational mechanism for the action of benzodiazepines [15,16], the direct enhancement of feeding by such drugs may involve modification of GABA functions in the "hunger-satiety" areas of the hypothalamus [1]

While increases in the amount of food deprivation tended to counteract the effectiveness of the drug, CDP produced a clear reduction in both rearing and ambulatory locomotion (squares entered) under the conditions of the present study Although these findings differ with the recently reported enhancement of "shuttle-box" locomotion by CDP [17,18], they may simply be further evidence for the situational specificity of such an effect Not only was activity assessed in an open-field arena in the present case, but more importantly, in the presence of an opportunity to eat, which has not been the case with other studies Furthermore, if one assumes that significant negative correlations in the action of CDP on feeding and activity reflect the potential for competing or incompatible behavioral effects, the present correlations (Table 1) suggest that the reduction in both rearing and ambulatory locomotion in the present case is due, in part, to the drug's enhancement of feeding and the increased time spent in such behavior This argument is further strengthened by the fact that the magnitude of the negative correlations between feeding and both measures of activity increased (along with an increase in potential for competing behavior) with the further enhancement of feeding produced by an increase in food deprivation While such a "competing response" hypothesis provides an explanation of multiple drug effects in behavioral terms, it does not preclude the possible role of central GABA mechanisms in mediating effects of CDP on activity, either indirectly through an anxiolytic action or directly by influencing motor systems To this point, however, attempts to define such central substrates have been disappointing [16]

The significant positive correlation in CDP's effects on rearing and ambulatory locomotion observed in the present case is not particularly surprising, although it is somewhat interesting to note that the interrelationship of these behaviors was relatively insensitive to differences in food deprivation Whereas rearing and ambulatory locomotion provide more independent sources of information in the assessment of d-amphetamine's effects under conditions identical to those described here [4], these measures would appear to provide somewhat more redundant sources of information in the present assessment of CDP's effects Such is suggested by both the magnitude and consistency of the correlations, which, again, may be due to the fact that both measures of activity were influenced in similar ways by the incompatible nature of concomitant feeding responses In any event, there would appear to be considerable value in employing multiple measures of general activity in assessing benzodiazepine effects, particularly in light of conflicting findings based, in part, upon how one defines such behavior

Finally, there would appear to be particular merit in a concurrent assessment of CDP's effects (or any drug's effect) on different behaviors as was done in the present case rather than an independent assessment of drug effects on different behaviors By permitting one to examine the interrelationships of a drug's action on different behaviors, such an experimental approach leads to more meaningful and realistic interpretations regarding the complex multiple action of the drug

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